The invention concerns heart cells with pathological characteristics, its development from embryonalen Stammzellinien and its use, in particular as in vitro model for heart illnesses (e.g. Arrhythmie, Hypertrophie, Ischämie). Areas of application of the invention are the pharmacology and the medicine (Kardiophysiologie).

Embryonale main cells (it cells), embryonale Karzinomzellen (input clutch cells) and embryonale germ cells (from primordial germ cells established EEC cells) differentiate, if they are cultivated in three-dimensional aggregates, so-called ?embryoid bodies? (EBs), into functional heart cells in vitro. These IT, INPUT CLUTCH or EEC cells concerns permanent Zellinien with, which are characterised by characteristics of undifferentiated embryonaler cells and which after development of the cell culture functional characteristics of differentiated cells mint.

For heart cells differentiated from it cells it could be proven that these regarding Genexpression and functional characteristics the specialized cells of the atrium, the ventricle and the pacesetter center to correspond. The heart cells react with characteristic chronotropic effects to kardiotrope agents (Wobus et al. 1991, deviation (1991) 48:173 - 182; Wobus et al., in vitro Cell. Dev. Biol. 1994, 425 [see patent specification of dd 299 439/85]). A recently developed procedure makes the computergestützte collection possible of chronotropic effects for routine investigations of pharmakologischer characteristics of heart-active connections (Pich et al., bio forum 20:536 - 540, 1997).

The choice of the culture conditions determines the efficiency of the heart cell differentiation as well as the differentiation sample, which are called by exogenous influences can the differentiation program modulated and which are reached differentiation induction into a certain heart cell type. Thus e.g. for the specific induction into ventrikuläre cells the time-dependent treatment with a certain concentration of the differentiation inductor Retinsäure (RA) was determined as effective differentiation induction (Wobus et al., J. Mol. Cell. Cardiol. 29, 1525-39, 1997).

The invention has as a goal to win by suitable choice of the differentiation conditions Zellinien with pathological characteristics which can serve for specific applications in the pharmacology (Screening of active substances) and the medicine (development of therapy strategies).

The invention is realized in accordance with the patent claims, that procedures which can be protected is characterised by the following steps:

1. It cells, input clutch cells or EEC cells from Vertebraten become in actually well-known way in embryo-similar aggregates, which differentiates so-called ?embryoid bodies? (EBs). It does not play a role whether embryoid the body differentiation takes place in the mass culture (?culture? measured) or in other culture procedures, e.g. in the hanging drop.
2. During the differentiation in accordance with the invention agents are added or selected culture conditions, which change the normal differentiation program going by that a changed development program is activated, so that heart cells are differentiated, which develop pathological characteristics. By choice of certain inductors (e.g. extracellular matrix factors and/or growth factors) pacesetter cells with arrhythmic action potentials can be e.g. predominantly differentiated. Other test conditions can predominantly lead a development, which is connected e.g. with a reactivation of the Expression of fetaler genes to the development from heart cells with characteristics of hypertropher cells.
3. The EBs is usually brought after a suitable time, after 5 to 7 days to the suspension culture, an adhäsive documents, where they attach and beside epithelial and other cells of areas of spontaneously pulsating heart cells attain full growth.
4. For the development of arrhythmically striking heart cells from it cells (any It-Zellinie can be used) the treatment of the differentiating EBs was used according to invention with a complex mixture from extracellular matrix proteins and growth factors (MATRIGEL) in the following procedure. Each procedure of the EB-differentiation can be used, which results in a sufficient high yield at spontaneously striking heart cells (see note under 1):
Expiration of attempt for the development of arrhythmically pulsating heart cells, schematically represented in fig. 1 with a selected example of 5 days of the EB-differentiation and plating.

A) It cells (e.g. n = 600 cells) of the line RI or other IT, INPUT CLUTCH or EEC-Zellinien are cultivated in the hanging drop in 20 mu l differentiation medium for the duration by 2-3 days and afterwards for the duration by 2 to 6 days (usually of 3-4 days) in suspension culture in bakteriologischen Petri plates. ISCOVE medium (Gibco, BRL) is supplementiert with L-Glutamin, not-essential amino acids, Monothioglycerol and 20% fötalem calf serum. In principle each medium is suitable, which results in a sufficient high yield at spontaneously pulsating heart cells.
b) 5 to 7 days old EBs (5 days old EBs are usually plated) are plated on fabric culture dishes, which were coated before with 10 mu g/ml Matrigel (e.g., MATRIGEL TM ?firs?, = basement diaphragm matrix, Collaborative Biomedical Products). The assigned Matrigel has in detail the following composition:

<tb><TABLE> Columns=2>
<tb>Title: Composition of Matrigel
<tb>Head Col 1: Component
<tb>Head Col 2: MATRIGEL

<tb>SubHead Col 1:
<tb>SubHead Col 2: Matrix
<tb>Laminin (mg/mg protein) <SEP>0,81
<tb>Collagen IV (mg/mg protein) <CEL AL=L>0,45
<tb><TSB AL=L>Heparan Sulfate<SEP>0,025
<tb> AL=L>Proteoglycan (mg GAG/mg protein)
<tb>Entactin (mg/mg protein) <CEL AL=L>0,12
<tb>EGF (ng/ml) <SEP>0,5-1,3
<tb>IGF-1 (ng (ml) <SEP>15,6
<tb>PDGF (pg/ml) <CEL AL=L>12
<tb><TSB AL=L>NGF (ng/ml) <SEP><0,2
<tb>TGF-beta (ng/ml) <SEP>2,3
<tb>% Protein-Gel<SEP>80
<tb></TABLE>

After few days of the culture differentiated cells, including spontaneously pulsating heart cells, attain full growth from the EBs.

A) The Matrigel treatment is 4 times repeated as over layering (?overlay?) during the following culture days in the distance from 1 to 3 days up to. The medium is removed and fresh differentiation medium, which contains Matrigel, is added the cultures. Already a unique gift of Matrigel led to an increase of the heart cell differentiation, an extended time sample of striking heart cells in the differentiation process as well as to an increase of the portion of arrhythmically pulsating heart cells, compared with such cultures, which were cultivated without Matrigel (= control). A four times repeated gift of Matrigel increased the efficiency of the heart cell differentiation as well as the portion of arrhythmically pulsating heart cells in the comparison as a check. Like that 28 days are present after plating 5 days old embryoid bodies (= to 5 + 28d, see fig. 2) of 48,0% arrhythmically pulsating areas, during in controls of only 12,5% arrhythmically pulsating areas - with substantially smaller heart cell differentiation efficiency (see fig. 2) - are present in the Matrigel treated cultures.

While in the control cells the portion of spontaneously striking heart cells goes approximately on the day 36 old after plating EBs 5 days against zero, the Matrigel induced cultures contain into approximately 60 to 70% the EBs spontaneously striking areas with heart cells, are called it are still cells with pacesetter activity available. These cells show however predominantly arrhythmic action potentials with partially high Pulsations frequencies.

Although in the control cultures also individual areas of spontaneously pulsating heart cells show arrhythmic Pulsationsfrequenzen, only the Matrigel induction results in the number of arrhythmically striking heart cells, necessary for the Screeninguntersuchungen of the pharmakologischen effect of anti Arrhythmika.

A) In attempts with isolated heart cells it could be shown that Matrigel the portion of the cells with pacesetter activity significantly increased and the differentiation in specialized heart cell forms restrained, so that the induction of heart cells into arrhythmically pulsating heart cells by Matrigel an incorrect development of the cells with pacesetter action potentials (more pacemaker action potential) is obviously the basis, is called it differentiates obviously fewer cells in specialized heart cells, e.g. Ventricle cells.
b) the collection of the flapping mode frequency takes place with the help of the Imaging system LUCIA ?Heart? (Nikon). Brightness differences are transferred in frequency samples and the values are computer-assisted digitized and seized.
C) the Matrigel induced heart cells are then used, in order to examine the effectiveness from antiarrhythmics to. The procedure corresponds to the procedure of a cumulative active substance addition described in the patent of dd 299 439/85 and a measurement of the effects normalizing the flapping mode frequency in principle with the help of the Imaging procedure (see to fig. 3 and 4).

Thus for the first time a heart cell model stands for order, which is suitable, to test the effect of antiarrhythmics in vitro.

Beyond that the procedure according to invention can be used, in order to obtain other pathological changes in the differentiated heart cells, in order to develop from it further into vitro models for rushing diseases:
The exogenous gift of Zytokinen, growth factors or hormones on differentiating EBs and/or the Überexpression in it cells of genes, the TNF alpha or other Zytokine and/or growth factors or hormones code, and following heart cell differentiation can lead to the development of heart cells with hypertrophen characteristics, which with a reactivation of the Expression of fetaler genes (e.g. beta - MHC, ANF, smooth muscle shares, C-fos, C-June among other things. ?immediate early gene?) accompanies.

Further know by Unterversorgung with nutrients (glucose withdrawal) or oxygen deficiency ischemic reactions in the differentiating heart cells to be released.

To be compiled the it cell differentiation model of pathological heart cells is efficient treatment strategies for the employment at humans suitably in the apron of the establishment of therapy strategies for ischemic changes cardiovascular (in addition, neural) fabrics and organs or of hypertrophen heart cells the meaning of intrazellulärer signal paths to be analyzed and.

The procedure is suitable in the future particularly if for heart cell differentiation embryonale main cells of humans, which can be established e.g. from primordial germ cells, are used.